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RESEARCH ARTICLE

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Evaluation of Growth Performance of Bioslurry Isolated Bacteria and their Application in *Ceratophyllum* sp. Fermentation for Sustainable Fish Feed

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ABSTRACT

Feed fermentation enhances nutritional quality, reduces feed costs, and mitigates pollution when processed with suitable microorganisms. This study investigated the growth and potential of three bacterial isolates from bioslurry—*Exiguobacterium aurantiacum*, *Bacillus indriensis*, and *Bacillus cereus*—as fermentation agents to improve the nutritional quality of *Ceratophyllum* sp. as a raw material for fish feed. Bacterial growth measurements conducted using spectrophotometry revealed a quadratic growth pattern with an R^2 value approaching 1. Peak growth was observed at 20.5–30.5h. The fermentation process, conducted over 24, 48, and 72h, significantly increased the protein and ash content while reducing the crude fiber and nitrogen-free extract (NFE) levels. The highest protein content was recorded after fermentation by *B. cereus* for 72h (34.80±0.007%), representing a 47.6% increase from the initial value. The most substantial reduction in crude fiber was observed after 72h of fermentation with *B. indriensis*, where it decreased from 14.38 to 4.26% (a reduction of 70.4%), indicating cellulolytic enzyme activity. The ash content increased, reflecting the release of essential minerals. Thus, fermentation using commensal bacteria from bioslurry is an effective strategy to optimize *Ceratophyllum* sp. as a high-nutrient and environmentally sustainable alternative feed source.

Keywords: Bioslurry, *Ceratophyllum* sp., Fermentation, Growth Bacteria, Protein

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INTRODUCTION

The aquaculture industry has experienced rapid growth in response to the increasing demand for farmed fish; however, it continues to encounter significant challenges concerning the availability of nutritious, affordable, and sustainable feeds (Jia et al., 2022). Commercial feeds, which primarily rely on fish and soybean meals as protein sources, are costly and compete with human food requirements (Moyo & Rapatsa-Malatji, 2023; Ragab et al., 2023; Magbanua & Ragaza, 2024). Consequently, there is a pressing need for alternative feeds derived from abundant local plants, such as *Ceratophyllum* sp., which is often regarded as a pest in fish ponds but has potential as a nutrient source. However, plant-based protein sources frequently contain anti-nutritional factors that impede digestibility and nutrient absorption in fish.

Fermentation with microorganisms, such as lactic acid bacteria (LAB), has been demonstrated to enhance the nutritional quality, reduce anti-nutritional factors and improve the growth and health of fish (Marti-Quijal et al., 2020; Chen et al., 2024; Gao et al., 2024; Neves et al., 2024). Therefore, utilizing fermented *Ceratophyllum* sp. as an alternative feed ingredient represents a promising strategy for supporting sustainable aquaculture. Feed fermentation employing microbes such as Lactobacillus, Bacillus, and yeast has been shown to diminish anti-nutritional factors (ANF) and increase the availability of essential nutrients (Neves et al., 2024). The incorporation of bacteria into fermented feeds has been shown to enhance digestibility (Jiang et al., 2023), thereby supporting growth and feed efficiency (Okoye et al., 2023). LAB decomposes complex carbohydrates, proteins and fibers in plant ingredients, rendering nutrients more accessible to fish.

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Bacteria commonly employed in the fermentation process include those of the genera *Lactobacillus* and *Bacillus* because of their capacity to produce extracellular enzymes that promote fish health (Banerjee & Ray, 2017) and enhance growth (Okoye et al., 2023). Bacterial isolates originating from specific habitats or local species generally exhibit a higher level of adaptation and demonstrate greater effectiveness in supporting the growth of cultured organisms (Wanka et al., 2018).

Bioslurry, a byproduct of the anaerobic fermentation of cattle manure into biogas, has potential as a probiotic source for sustainable fish feed. Research indicates that solid bioslurry can enhance the growth and feed efficiency of tilapia (*Oreochromis niloticus*) (Jamaluddin et al., 2025), whereas liquid bioslurry increases the dissolved protein content in feed (Masriah et al., 2024) and improves the survival rate of milkfish (Zaenab et al., 2022). Our previous research identified several *Bacillus* strains with enzymatic activity in bioslurry isolates (Zaenab et al., 2025). These findings align with those of prior studies that confirmed the efficacy of *Bacillus* spp. in enhancing digestion, gut health, and fish performance (Shija et al., 2025).

The success of the fermentation process is largely contingent on the type of bacteria and the duration of fermentation, as both factors influence the enzyme activity produced. Each bacterium exhibited a distinct pattern of growth and enzyme production over time. Therefore, this study aimed to utilize various types of bacteria from bioslurry to ferment *Ceratophyllum* sp. and evaluate the optimal fermentation duration to produce an alternative protein source for sustainable fish feed.

MATERIALS & METHODS

Bacterial Preparation

Bacteria (*E. aurantiacum, B. idriensis,* and *B. cereus*) isolated from the bioslurry were cultured on TSA medium and subcultured by incubation for 24h at 37°C before use (Madigan et al., 2018).

Bacterial Activation

Each bacterial isolate grown on TSA medium was subcultured by inoculating a loopful into TSB and incubating at 37°C for 24h to reactivate cell metabolism (Madigan et al., 2018). A total of 1mL of culture was serially diluted in sterile 0.85% NaCl solution until a dilution of 10^{-5} was reached. All procedures were performed aseptically using sterile pipettes, and each dilution was mixed thoroughly before being used for further analysis (Cappuccino & Welsh, 2017).

Preparation of Fermented Gosse

Ceratophyllum sp. collected from ponds was dried without direct exposure to sunlight to preserve its nutritional content and then ground into a fine powder. The powder was then fermented using *E. aurantiacum*, *B. idriensis,* and *B. cereus*, each inoculated with 1000mL of culture (1×10^5 CFU/mL) per 1kg of powder. The mixture was homogenized, packed in black polyethylene nylon plastic, and incubated anaerobically at 37°C for 24, 48 and 72h.

Experimental Design

This study used a completely randomized design (CRD) with a 3 × 3 factorial pattern consisting of three bacterial isolates from bioslurry and three fermentation durations. Each treatment combination was repeated thrice, resulting in 27 experimental units (Table 1).

Table 1: Research Design

Type of bacteria	Nutritional Composition of Feed Based on Fermentation Time								
	a (0h)			b (24h)			c (48h)		
A (*E. aurantiacum*)	Aa1	Aa2	Aa3	Ab1	Ab2	Ab3	Ac1	Ac2	Ac3
B (*B. idriensis*)	Ba1	Ba2	Ba3	Bb1	Bb2	Bb3	Bc1	Bc2	Bc3
C (*B. cereus*)	Ca1	Ca2	Ca3	Cb1	Cb2	Cb3	Cc1	Cc2	Cc3

Test Parameters

Bacterial Growth Curve Measurement

Bacterial growth was quantitatively measured using a spectrophotometer at a wavelength of 540nm (OD_{540}). A total of 1mL of culture was aseptically placed into a sterile cuvette and measured against a TSB blank. Observations were conducted every two hours from the 10th to the 32nd hour to monitor the growth curve.

Chemical Analysis of *Ceratophyllum* sp.

Chemical analysis was performed to determine the nutrient content of *Ceratophyllum* sp. before and after fermentation, including protein, carbohydrates, fat, crude fiber, ash and Nitrogen-Free Extract (NFE) at each fermentation time, using the standard AOAC (2005) method.

Data Analysis

Nutritional data were analyzed using two-way factorial analysis of variance (Two Way ANOVA) to evaluate the interaction between the type of bacteria and fermentation duration. The W-Tukey post-hoc test was performed at $\alpha=0.05$ if a significant difference was found. Interpretation was conducted using Orange Data Mining.

RESULTS AND DISCUSSION

Bacterial Growth

The growth of three bacterial isolates, *E. aurantiacum*, *B. indriensis* and *B. cereus*, was analyzed spectrophotometrically at 540nm. The specific standard curves for each isolate showed differences in the growth patterns and cell density. The relationship between absorbance and cell number is depicted by linear regression equations (Fig. 1).

Fig. 1 shows standard curves illustrating the relationship between absorbance values and log cell numbers of the three bacterial isolates, which displayed a positive linear correlation as well as viability and metabolic stability, as indicated by the high coefficients of determination (R^2) *E. aurantiacum* (0.9656), *B. indriensis* (0.9399), and *B. cereus* (0.94380). This indicates that high growth activity correlates with the capacity for enzyme production, which serves to hydrolyze complex components into more digestible forms (Padhan et al., 2025). Therefore, the isolates *E. aurantiacum*, *B. indriensis*, and *B. cereus*, each with an $R^2>0.93$, have the potential to serve as reliable fermentation agents for improving the

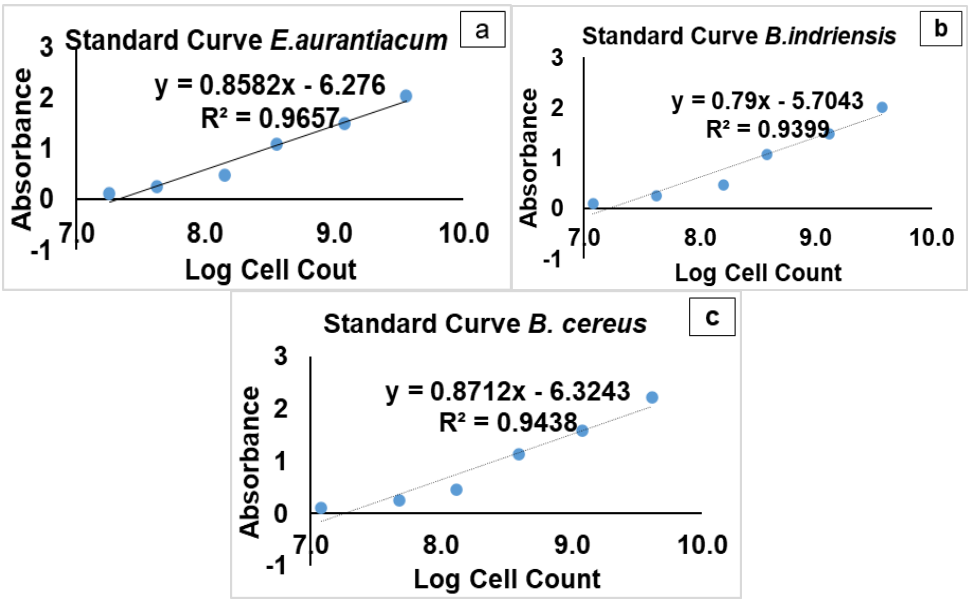

Fig. 1: Standard curve of the linear equation for the relationship between absorbance and bacterial cell number of *E. aurantiacum* (a), *B. indriensis* (b), and *B. cereus* (c).

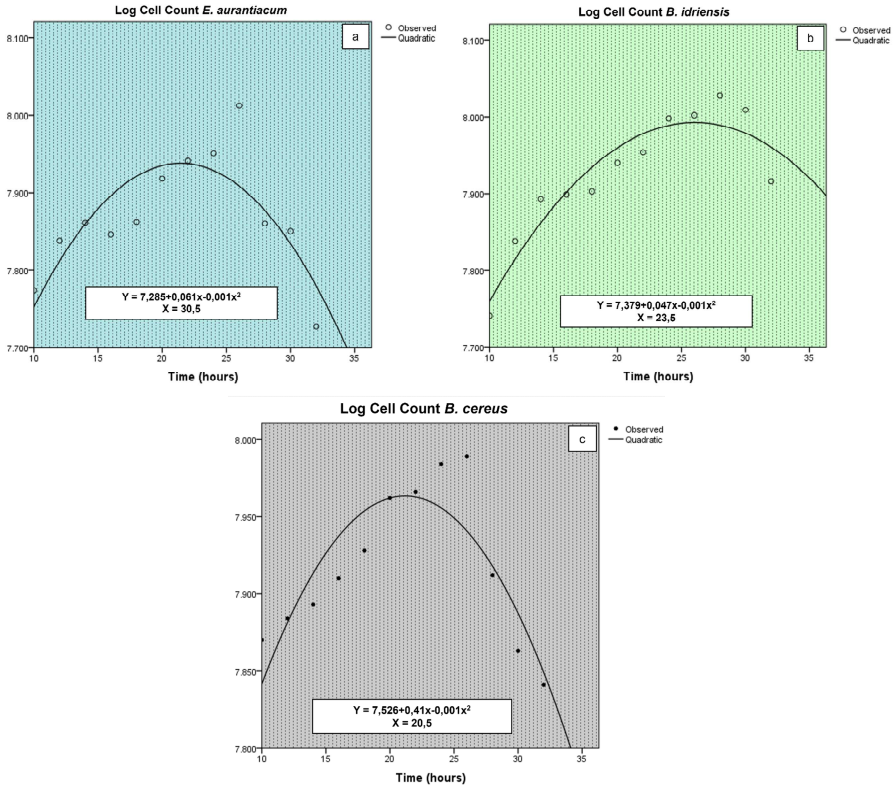

Fig. 2: Growth patterns of *E. aurantiacum* (a), *B. indriensis* (b), and *B. cereus* (c) at each growth interval are shown.

nutritional quality of feed ingredients by increasing digestibility and nutrient availability (Beal et al., 2020; Sawant et al., 2025).

The results of absorbance conversion through the standard curve indicated that all three bacteria followed a characteristic growth pattern consisting of the lag, exponential, stationary, and decline phases, as shown in Fig. 2.

Fig. 2 shows the growth dynamics of each bacterium during incubation, modeled as quadratic curves of log CFU/mL against time. The growth peak was reached at 30.5h (*E. aurantiacum*), 23.5h (*B. indriensis*), and 20.5h (*B. cereus*), reflecting the exponential phase prior to transitioning into stationary and decline phases. This pattern demonstrates the typical stages of bacterial growth, from the lag phase to the death phase. The observed growth patterns indicate that each isolate has a different optimum time for reaching peak growth, which is important for determining the most effective fermentation period for each isolate. According to Madigan et al. (2021), bacterial growth in culture media generally forms a sigmoidal or parabolic curve, reflecting the dynamics of microbial populations throughout their life cycle. The peak log CFU/mL value represents the maximum viability and highest biosynthetic activity, making it a crucial reference for fermentation applications (Sawant et al., 2025).

The suitability of the quadratic regression model in specifically describing the distribution patterns of each bacterial growth can be observed in the contour plot diagram in Fig. 3.

The distribution of each bacterium's growth in a two-dimensional contour diagram based on the incubation time and log cell count (Fig. 3). The colored zones indicate the growth phases: blue (lag phase), green (exponential phase), and yellow (stationary–decline phase). The results of this study show that the three bacterial isolates have different optimal growth times, with *E. aurantiacum* reaching peak growth between 25 and 30h, *B. indriensis* between 20 and 30h, and *B. cereus* before 30h. The pattern of data distribution on the two-dimensional contour ellipses shows that the highest growth activity for all isolates generally occurred within the 20–32h interval. These findings are consistent with the assumption that the exponential growth phase is the most metabolically active phase, during which enzyme and bioactive compound production is at its highest.

Their abundance determines the involvement of bacteria in maintaining host homeostasis, and changes in cell count can potentially affect the stability of the host microbial ecosystem (Kim et al., 2024). Quantification and monitoring of bacterial growth are essential in microbiological studies, particularly for evaluating metabolic activities, such as enzyme production. Optimal growth often correlates with high levels of enzyme production, which has potential applications in feed biotechnology (Padmavathi et al., 2018). Therefore, measuring bacterial growth not only reflects population dynamics but also serves as an important indicator for assessing the functional capacity of bacteria in fermentation processes and improving feed quality (Madigan et al., 2021; Padhan et al., 2025).

The Effect of Bacterial Type and Fermentation Time on the Proximate Nutrition of *Ceratophyllum* sp.

Fermentation of *Ceratophyllum* sp. increases its nutritional value, making it a more efficient and sustainable alternative to fish feed. This process breaks down complex compounds into forms that are more easily absorbed by the body. Proximate analysis showed that both the type of bacteria isolated from the bioslurry and the length of fermentation significantly affected the nutrient composition of *Ceratophyllum* sp., as shown in Table 2.

Ceratophyllum sp. is an aquatic plant with high productivity and rapid growth (Aparicio et al., 2021). Its high protein and NFE content (Table 2) has the potential to be degraded by microorganisms and is suitable for use as sustainable fish feed (Hussain et al., 2024; Usmanbaha et al., 2025), while also contributing to the reduction of aquaculture waste. The high crude fiber content results in low digestibility of *Ceratophyllum* sp. when used directly as a feed material. Biotechnological treatments, such as fermentation with enzyme-producing microorganisms, are needed to improve digestibility and nutritional value.

Fermentation of *Ceratophyllum* sp. with *E. aurantiacum* increased the protein content by 36.6% after 72h. The highest crude fat content was reached at 24h, whereas crude fiber decreased significantly by up to 65.72%. The decrease in nitrogen-free extract (NFE) and the increase in ash content indicate carbohydrate degradation and mineral release during fermentation. *E. aurantiacum* was isolated from bioslurry (Zaenab et al., 2025), which has shown enzymatic activity and plays a role in energy metabolism and the bioconversion of organic materials (Rui et al., 2023).

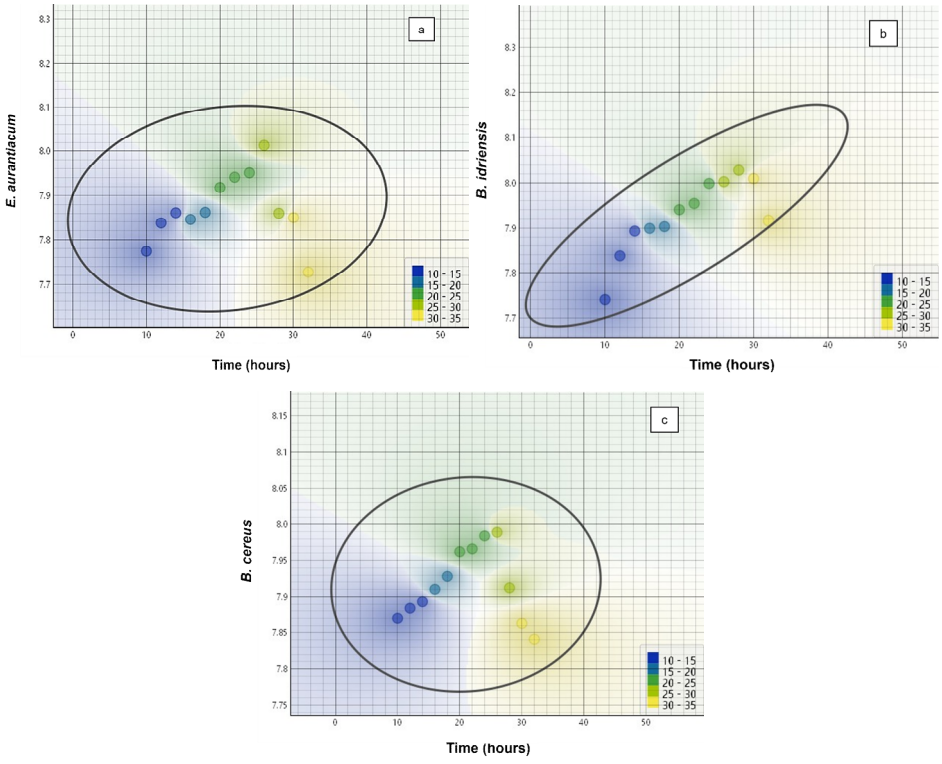

Fig. 3: Contour plot diagram of the relationship between incubation time and log of bacterial cell counts for *E. aurantiacum* (a), *B. indriensis* (b), and *B. cereus* (c).

Table 2: The effect of bacterial type and fermentation time on the proximate nutrition of *Ceratophyllum* sp. is presented in the following table

Treatment		Parameter (±std)				
Bacterial species	Fermentation Time (h)	Protein	Crude Fat	Crude Fiber	NNF	Ash
initial		23.58	0.23	14.38	42.58	19.24
E. aurantiacum	24	22.53±0.056a	0.76±0.030ab	9.88±0.045a	38.15±0.030a	28.69±0.035a
	48	29.13±0.035b	0.64±0.020a	8.95±0.035b	33.75±0.075b	27.53±0.050b
	72	32.22±0.510c	0.72±0.010b	4.93±0.020c	30.84±0.030c	31.30±0.035c
B. indriensis	24	32.29±0.105d	0.75±0.035cd	7.75±0.035d	31.34±0.045d	27.89±0.015d
	48	33.39±0.070e	0.72±0.010c	4.70±0.200e	32.02±0.020e	29.17±0.080e
	72	33.66±0.220f	0.68±0.010d	4.26±0.050f	29.02±0.010f	32.37±0.020f
B. cereus	24	24.13±0.060g	0.32±0.040ef	11.92±0.010g	38.40±0.030g	25.23±0.010g
	48	33.87±0.020h	0.43±0.010e	10.58±0.030h	28.81±0.020h	26.30±0.200h
	72	34.80±0.007i	0.44±0.014f	8.20±0.021i	27.20±0.084i	29.15±0.028i

Values (mean+SD) bearing different letters in the same column indicate a highly significant (P<0.01) difference.

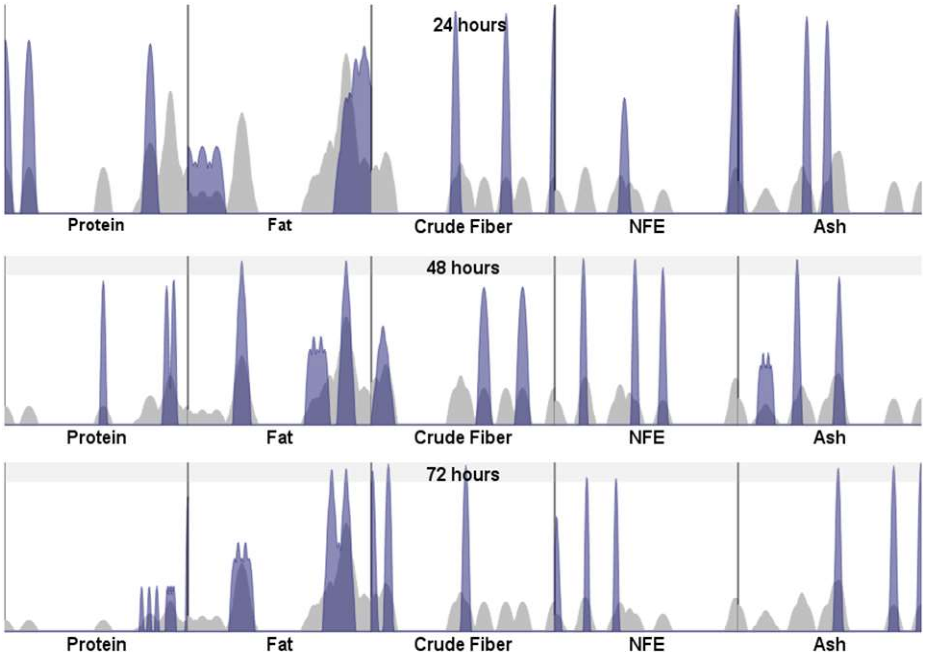

Fig. 4: *Radar-style area chart* comparing the nutritional composition of fermented goose at different fermentation durations.

The isolate *B. indriensis* resulted in a 42.75% increase in protein and a 70.36% reduction in fiber after 72h of fermentation of *Ceratophyllum* sp., indicating intense enzymatic activity. The crude fat content remained stable, whereas the ash content peaked at 48h, reflecting efficient substrate utilization and mineral release from the fermented biomass. *B. indriensis* has functional potential as a fermentation agent for improving the nutritional quality of feed ingredients such as *Ceratophyllum* sp. through the production of enzymes with high and stable biodegradation capability throughout the fermentation process (Du et al., 2017).

B. cereus exhibited the highest fermentation efficiency, with protein levels increasing by up to 47.60% at 72h. The most drastic reduction in NNF was 36.06%, reflecting the utilization of carbohydrates as the main energy source. The decrease in crude fiber and the increase in ash content also support the role of this bacterium in modifying the nutrient composition during fermentation. *B. cereus* has high potential for biotechnological applications, particularly in fermentation and feed formulations, because of its ability to produce enzymes such as α-amylase, protease, lipase, and cellulase, which assist in breaking down complex nutrients (Liu et al., 2016). In addition, *B. cereus* serves as an effective probiotic that enhances antioxidant enzyme and metabolic activities in tilapia (*O. niloticus*) and has been applied in aquaculture to support fish health (Hao et al., 2014). Overall, all three isolates were effective in modifying nutrient composition, but *B. cereus* and *B. indriensis* produced the most optimal results in increasing protein content and reducing crude fiber, making them leading candidates for fermentation applications in the processing of nutritionally valuable fish feed (Yang et al., 2020).

Fig. 4 shows the dynamics of the nutritional composition of *Ceratophyllum* sp. based on fermentation time. In general, the intensity (density of the colored area) increased with fermentation time.

The results show that the duration of fermentation plays an important role in determining the nutritional quality of *Ceratophyllum* sp. The longer the fermentation, the greater the enzymatic activity that breaks down macromolecular components, such as proteins and carbohydrates (Fig. 4). Therefore, optimizing the fermentation duration is crucial to achieve a balance between increased nutritional value (Li et al., 2021).

At 24h, the protein and fat contents began to increase but had not yet reached their peak values. At 48h of fermentation, there was a sharp increase in protein and fat, as well as a more significant decrease in crude fiber than at 24h. Meanwhile, at 72h, peak activity was observed in the protein and crude fat fractions, with maximum reduction in

crude fiber and NFE, indicating the degradation of complex compounds into simpler and more digestible forms. The sharper and more intense nutrient distribution in the 72h fermentation shows maximal enzymatic and metabolic activity by bacteria, supporting previous quantitative findings that protein content increases significantly, while crude fiber and NFE decrease. This is consistent with the microbial fermentation mechanism of breaking down polysaccharide compounds and increasing the availability of organic nitrogen (Chan et al., 2023; Bezerra and Fonseca, 2023; Islam et al., 2024). In addition, the increase in ash content indicates the release of mineral elements, which is important for supporting fish growth. The increase in ash content after fermentation suggests that the microorganisms used, such as *B. cereus*, *B. indriensis*, and *E. aurantiacum*, play a role in degrading the complex structure of the plant and releasing bound minerals such as calcium, magnesium, and phosphorus (Li et al., 2021). Therefore, fermentation for 72h demonstrated the highest efficiency in improving the nutritional quality of *Ceratophyllum* sp. as a more functional and nutritious fish feed ingredient.

Conclusion

Fermentation of *Ceratophyllum* sp. with bioslurry bacterial isolates, *Exiguobacterium aurantiacum*, *Bacillus indriensis*, and *Bacillus cereus*, significantly increased the nutritional value of feed ingredients. Fermentation for 72h with *B. cereus* produced the highest protein content at 34.80±0.007%, a 47.6% increase from the initial value (23.58%). *B. indriensis* showed the most significant reduction in crude fiber, from 14.38% to 4.26±0.050% (a decrease of 70.4%), indicating a strong cellulolytic enzyme activity. The highest ash content was recorded after 72h fermentation with *B. indriensis* (32.37±0.020%), reflecting the release of essential minerals during the fermentation process. Bacterial growth measured using the standard curve showed a positive correlation between cell density and increased nutrient content, indicating that bacterial metabolic activity directly contributes to fermentation efficiency. Therefore, this approach shows promise for optimizing *Ceratophyllum* sp. as a high-value, economical, and sustainable alternative feed ingredient in aquaculture.

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Data Availability: All the data generated during the study are present in the article.

Ethics Statement: No ethical approval was required for this study.

Author's Contribution: The authors have made substantial contributions to this stage of the research and collaborated to ensure the quality and objectivity of the study. SZ conceptualized the study, collected and analyzed the data, and wrote the manuscript. Z, S, and KN contributed to the development of the methodology, data curation, drafting of the manuscript, and its revision. All authors have read and approved the final version of this manuscript.

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